The Role of Biological Sampling in Investigations of Indoor Air Contamination

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Molds are everywhere on our planet since they and other fungi compost organic substances during biological recycling of molecules. Molds need moisture to grow in the outdoor environment as well as indoors. Molds found indoors in dry buildings do not grow there, but are tracked in on shoes, or enter through openings in the building. If sampling procedures were done indoors in dry buildings molds would be present, but would present no health problems to most occupants. To ascertain that molds spores found indoors did not grow there, they should be of the same nature (genus and species) as those commonly found outdoors. They should also be at, or more likely, in fewer number than those found outdoors. There are additional indicators whether mold is growing indoors. For instance, mold odors come from living, growing molds since they are the metabolic products of living organisms. Therefore, if you smell mold, then mold is growing. Mold that is growing will also have chain of spores or conidiae (fruiting bodies) that can be seen under the microscope.

If mold is seen or smelled in a building, mold is present. Visible mold found growing indoors it is always due to excessive moisture. If the source of moisture is found and remedied, and the extent of contamination is known, and cleaned up properly, there is no need to conduct sampling.

Mold grows indoors when there are moisture problems. The task then is to find the source of moisture and to remedy it. Common sources of moisture are plumbing leaks, which may be in hidden spaces under kitchen or bathroom cabinets or in walls, or moisture intrusion may come from outside. Roofs, walls, windows and foundations are common areas of moisture intrusion. Water will penetrate most exterior building materials, but diverting water away from vulnerable points of the structure through flashing, and providing a drainage plane for moisture that has penetrated to leave the structure, can prevent moisture intrusion that will result in mold growth. Proper foundation drainage will prevent moisture intrusion into basements or crawl spaces. When prevention of moisture intrusion fails, prompt attention to, and remediation of leaks is required to prevent mold growth.

Everyday activities such as showering, bathing, washing clothing or dishes, cooking, and other activities put moisture in the air, and because modern construction tends to be tight for energy conservation, the accumulation of all this moisture indoors can also be a source of mold growth. Human beings also contribute about a quart of water per day each by breathing (when not active; more if active). This moisture also needs to be removed. If not removed, the air will become saturated with moisture, particularly near and on cold surfaces, and water will condense leaving a film of moisture in which mold will grow. Mold growth on interior surfaces, especially on outside walls and on windows that are cold, is often an indication of inadequate ventilation. Adequate ventilation through heating/cooling systems, or through the use of fans in high moisture areas like kitchens or bathrooms, is necessary to prevent moisture problems and mold growth. An air gap between cold outside walls and furniture such as dressers or bookcases also helps air to circulate and prevents condensation which could result in growth.

When buildings are suspected or known to have mold contamination, there is often a need expressed to perform biological sampling to determine the nature of the contamination. In fact, biological sampling of various kinds is only one tool among many that can be used. Sampling may not be necessary, depending on what can be learned through other, less costly, ways of assessing such contamination.

Often problem buildings come to the attention of health officials because there are complaints from the occupants of the building. Such complaints are often the first information that needs to be assessed. This may entail actual conversations with the complainant to determine where and when complaints of illness or discomfort occur. Such information can determine whether the complaints are building-related. If the complaints are building-related, answers to such questions often lead the investigator to potential problem areas. Conversations with occupants, maintenance personnel, custodians, and staff who have been in the building over time, may also lead to important institutional history that can lead the investigator to look for moisture sources that might otherwise be overlooked.

An actual assessment of the complaints, or further look at medical records if the complainant has seen a doctor, may also help the investigator to distinguish among possible sources of health effects (including other exposures), and to distinguish between chemical and biological causes of illness, or to determine if ventilation problems may be behind the complaints.

Under certain circumstances, sampling may be a tool an investigator may choose to gain additional information. When a decision to perform sampling is made, it should only be done to answer a specific question. Sampling also should not be done without a specific written sampling plan, since improper sampling will yield little or no information helpful to the investigation. Even when investigators are using sampling to address answerable questions (testing hypotheses), and are working from a well-thought-out sampling plan, results from bioaerosol monitoring can be inconclusive and misleading.

The question being asked will determine the kind of sampling that should be done. For instance a suspect discoloration on a wall can be sampled with a tape lift that is examined under a microscope to determine whether it is soot or mold.

Dust sampling may help to address how long the problem of mold growth has existed. Air sampling may reveal whether spores are present in air people breathe, or to determine whether there is a pathway for mold to travel in air from a hidden reservoir in a wall or chase to occupants of rooms. Sampling for microbial volatile compounds can help to determine whether a contamination problem is small or extensive. Analysis of dust, building materials or mold spores and fragments can determine the presence of mycotoxins (poisons produced by a number of molds). Air and surface sampling can be used to determine the efficacy of remediation and clean-up, or to determine "how clean is clean?"

Sampling for spores using tape-lifts on surfaces, or one of various spore traps can give an initial indication whether broad classes of mold spores are present on surfaces or in the air. Spores are identified by their appearance under a microscope. No specific identification (except for molds that have few species within a genus such as *Stachybotrys*) can be done through these analyses. Spores of the species within genus *Aspergillus* produce spores that are generally indistinguishable from each other or from those species within the genus *Penicillium* (these are frequently reported as "*Aspergillus/Penicillium*-like or "Asp/Pen-like"). Quantitation of spores varies with the spore-trapping device being used, and does not translate from one to the other since exact efficiencies are not known. Efficiencies of samplers have been compared to each other, but how realistically the "best" samplers characterize exposure of occupants is unknown.

In order to identify molds to the species level, or even to distinguish closely related genera such as Aspergillus and Penicillium, viable sampling must be done. This involves various means of capturing mold spores into liquid or solid growth media. The samples may be serially diluted, and cultured under laboratory conditions, and then examined using various biological and chemical techniques to determine the species of the mold captured from the air. The process takes time, however, both for the molds to grow on culture medium to large enough numbers to be visible as colonies, and for the identification processes. Labs certified by the U.S.EPA can employ new molecular biological techniques polymerase chain reaction or PCR that can rapidly identify most molds to the species level from both spore-trapping and viable means. However, the technique cannot distinguish between live and dead spores. Since dead spores retain their allergenicity and toxicity for long periods of time, distinction of viability could be important to an investigation of health effects.

Whether or not any of these sampling techniques is applied depends entirely on the question being asked. Questions range from the very simple, such as "Is it mold or not" to very complex such as "Is there exposure to toxic mold." The first is easily determined through a tape lift. The second requires much more information and much more complicated and expensive testing. At best it may not be able to quantify exposure, but only show an association between exposure and effect. The reasons for this are

- Determination of exposure requires that one knows the extent and duration that a person has been impacted.
- Determination of effect also requires that one know the nature and extent toxic impact of substances to which an individual is exposed
- Both of these require that sampling methodology and other analyses can determine the nature, extent and duration of exposures.

Molds that disseminate their spores through the air do so in "blooms" which are episodic, and whose periodicity is not predictable. Continuous air sampling is rarely done, because it is expensive. Much more commonly, samples are taken in a few locations in a building (and outdoors for comparison), at one or more times during one day, or in some case multiple days. The sampling event can easily miss a period of bloom for one or more mold species. Other molds, such as *Stachybotrys*, are wet and slimy when they are growing, yet when dry, are easily aerosolyzable and inhalable when disturbed through activity within the building. Since moisture intrusion is often a factor of weather, many damp and moldy buildings have periods of time when they can dry out, along with the molds contaminating them. Time of sampling becomes important for this reason as well. Human activity levels in buildings vary, and can affect levels of particles in the air. Again, time of sampling becomes important in trying to assess exposure.

The nature of sampling devices is also a problem in trying to quantify exposure. The various spore traps have differing efficiencies and capture differing amounts from the same air in simultaneous sampling. There is not a methodology for mathematically translating the results of such sampling from one kind of device to the next. Spore trapping does not allow a full spectrum of identification, since analysis is limited to what can be visually distinguished. The problem is even more complex in attempting to compare non-viable (i.e., spore trap) with viable sampling. Viable sampling in itself has limitations, which vary depending on the device and media used. Not all viable spores physically captured will grow. The sampler itself may injure or kill some spores, or may plant it too deep into the medium for growth to occur. The investigator may not guess correctly what the expected molds are and may not provide a growth medium that allows all molds in a sample to grow. Molds differ in their nutritional requirements and environmental conditions, such as water content and temperature. Molds inherently have different grow rates, and fast growers can overgrow slow growers. Molds also can inhibit growth of competitors by excreting molecules such as antibiotics and toxins into the growth medium, so that while a particular species has been captured, it won't grow to a countable colony.

Assessment of exposure to the toxic substances that some mold species produce is particularly problematical. There are currently few methodologies for detecting mycotoxins in human tissues or bodily fluids. Separating toxins acquired from foods from those acquired from indoor or outdoor exposures, is not possible.

Assessment therefore depends on whether or not one measures and detects any or the entire spectrum of toxins a mixture of mold spores that a person may inhale or have skin contact with. (Such assessment assumes that the investigator has properly characterized the mixture of molds growing, airborne, and producing toxins). However, spore capture and isolation is not the only part of exposure that needs to be characterized. Molds that produce mycotoxins exude these poisons into the substrate or environment in which they are growing. The reason why molds produce toxins in the first place is that they are useful in inhibiting or killing off competitors that share the same ecological niche. (Penicillin antibiotics were first discovered by observation of the ring of bacterial growth inhibition around *Penicillium* contamination in the medium on which the bacteria were growing). Recent research has shown that building materials and dust resulting from such materials can contain toxins in the absence of spores. Spore sampling alone therefore may not be sufficient to characterize mycotoxin exposure. Toxigenicity is best determined directly from the sample taken in a building. Detection, however, requires a sufficiently large sample. Culturing the sample to amplify growth is not very effective. When toxigenic molds are captured they can be shown to be produce mycotoxins in the building environment. Growing those same molds in pure laboratory conditions without competitors allows the molds to stop producing mycotoxins after a few generations of growth.

Air always contains a mixture of contaminants. In damp buildings, multiple species of molds, bacteria, other microbes and their products can be found in addition to sources of chemical contaminants such as volatile and semi-volatile compounds and particles. Focusing on a particular species such as *Stachybotrys* or *Aspergillus* does not allow for a sufficient analysis of bioaerosols that might be impacting occupants' health.

However, finding particular species, such as *Stachybotrys,* or *Aspergillus versicolor* or *A. sydowii* is still significant. Such organisms are an indicator of long term or severe moisture problems. Presence of such an organism does not necessarily indicate exposure. Such a question needs to be answered by examining other information such as health effects, sensitivity, and sampling that can determine contact of an ill person to a contaminant. Since health is related to total exposure, focusing only on such organisms to determine whether or not conditions are safe for occupants gives a skewed and potentially erroneous picture. Absence of such organisms in a sample, in the face of other evidence indicating mold presence, does not guarantee their absence in the environment, nor does it guarantee the absence of other molds that could be problematical. Absence of evidence does not indicate evidence of absence.

Because mold exposure can have health consequences, including allergy, irritation, odor effects and toxicity, evidence of mold contamination needs to be taken seriously. One does not necessarily need to know the identity of the culprit to solve and address the problem of mold contamination. A good investigation by building and health professionals can often uncover the reason and source for such contamination. Investigators use their knowledge of building construction and function, heating, ventilation and air conditioning systems, the processes of ventilation themselves, and knowledge about mold, its growth habits and requirements, as well its potential for association with health problems to locate growth. Remediating the moisture problem, and cleaning up mold contamination to prevent further exposure, is the prudent course of action. With the potential health consequences known, prudent public health practice is to prevent further exposure.

Sampling for biological contaminants such as bacteria, mold spores or fragments, mold products such as volatile organic compounds or toxins, on surfaces, building materials, dust, or in air is only one tool in investigating mold problems in buildings. There are also many levels of such sampling, which can each answer only limited questions. Sampling should never be the first course of action. Unless this tool is used to answer questions, the results will not have meaning.

Looking for moisture intrusion, and determining whether there is functioning and adequate ventilation in a building should always be done before a decision to sample for biological contamination is made. Measurements of humidity in the air, or moisture measurement in a wall, or concrete slab, are also useful determinants of the potential for mold growth and should precede actual biological sampling. It must be recognized that even when care is taken in making sampling decisions, and a good sampling plan is being used, sampling may give inconclusive and possible misleading information. Sampling needs to be used when required, in the context of other tools. A walk-through by a competent building investigator, together with solicitation and analysis of complaints and symptoms by knowledgeable public health or medical personnel can often uncover sources and an association with the sources of biological contamination.

Determining whether and how much mold contamination exists, and determining whether there is a health impact on occupants, requires the cooperation of knowledgeable people. Persons who are suffering symptoms that seem to be building-related should report their experience to decision makers in the building. The knowledge of occupants of when and where they experience symptoms is essential to an investigation, so avenues of information reporting must be present and complaints must be taken seriously. Occupants also often have institutional history of building changes and remodels, or past moisture intrusions or catastrophic water accidents that will help investigators find sources of moisture and contamination. Tracking of illness complaints of occupants is also essential to detect temporal or spatial relationships to contamination, or to rule out episodes of infectious illness not related to building conditions.

Knowledge of symptoms related to exposure to contaminants, and knowledge of other potential sources of symptoms is required to rule out causes other than moisture–related biological contaminants. Lack of ventilation due to design flaws or changes in air conditioning systems can concentrate chemical air contaminants whose symptoms overlap with irritation, allergy or toxicity associated with indoor molds and bacteria.

Knowledge of building structure and function, and what determines pressure difference and air flows is essential to tracking distribution of contaminants. Understanding moisture dynamics in structures is also needed, if pathways of intrusion are to be traced and remediated.

Buildings can be kept dry and clean, if they are designed for the climate in which they are to function, are properly sited to divert moisture from their foundations, are carefully built to specifications, and are promptly and properly maintained. Having knowledgeable staff that have access to decision makers when prompt intervention to correct moisture problems is needed is essential to preventing problems that result in mold growth. Even with catastrophic moisture accident, prompt drying and cleaning can prevent mold growth. Such intervention is usually far less costly than investigation, remediation and clean-up after mold-growth has occurred. More importantly, it prevents impacts on occupants' health, which is in itself a great cost savings.